Innervation of the Rat Anterior Abdominal Wall as Shown by Modified Sihler's Stain

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Key Words

Sihler's stain technique · Abdominal wall muscle innervation

Abstract

Objective: The purpose of this study was to use the modified Sihler's staining technique to demonstrate detailed distribution of the rat anterior abdominal wall nerves and test the value of Sihler's technique in demonstrating such a complex muscle-nerve relationship. **Materials and Methods:** The anterior abdominal walls of 5 Wistar rats were isolated by making a deep incision from the costal arches on each side down to the inguinal region and processed using a modified Sihler's stain technique. **Results:** This technique was successfully applied to visualize the innervation of the anterior abdominal wall muscles of the rat. The segmental nerves of T₆–L₁ and their terminal branches were shown and possible motor and sensory fibers identified. **Conclusions:** This technique is valuable in understanding the complex nature of final branching of the nerve endings, and it may be useful for studying experimental nerve models.

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Introduction

Anatomical dissection has been the most common method for tracing nerve fibers to their fine intramuscular terminal branches. The intramuscular branches, however, cannot easily be revealed by direct dissection. Such dissections, even under the microscope can damage muscle fibers and disrupt the normal anatomical relationship between muscle and nerve fibers. Computer reconstruction of serial histological sections has also been used as an alternative method for tracing nerve supply. Also, gold chloride staining has been applied by many to visualize nerves. Even with this method, nerve fibers were visible only superficially, and tracing their branches in deep sections was not possible [1, 2].

Charles Sihler [3] introduced a method for staining nerve spindles in 1895, but the work remained largely forgotten until recently. The technique makes whole specimens transparent so that the intramuscular nerve supply appears in its original three-dimensional state. Consequently, tracing the complex structure of terminal nerve branches becomes easier. Many researchers have reported modifications of Sihler's technique for identifying innervations to various organs [4–7].

The muscles of the rat anterior abdominal wall, namely the external oblique, internal oblique and transverse, form three thin aponeurotic layers which eventually en-

velopes the rectus abdominis ventrally, similarly to those of humans. These muscles are supplied by lower thoracic spinal nerves, iliohypogastric and ilioinguinal nerves. There are 34 spinal nerves in the rat: 8 cervical, 13 thoracic, 6 lumbar, 4 sacral and 3 caudal. Morphologically, the pattern of anterior abdominal wall innervations is like that of humans. However, a communicating branch of the 13th thoracic nerve frequently joins with the first lumbar nerve. It divides into two branches; a superior one (the iliohypogastric nerve), which may be joined by a branch from the 13th thoracic, and an inferior one (the ilioinguinal nerve), which is somewhat variable. In some cases, these two branches may form a single trunk for a short distance, or the inferior branch may unite with a branch from the second lumbar nerve [8, 9]. The iliohypogastric nerve first gives cutaneous branches (lateral and ventral) for the anterior abdominal wall, then follows its course and eventually divides into cranial and caudal branches [10]. The muscles of the anterior abdominal wall and their nerve supplies are fairly complex and the gross dissections of branches of related muscles are somewhat difficult [11]. Furthermore, when tracing nerve branches deeper into the muscles they innervate, preserving one branch often necessitates unwanted destruction of other nerves to the same structure [12]. We designed this study in order to demonstrate detailed distribution of the anterior abdominal wall nerves of the rat and tested the value of Sihler's technique in such a complex muscle-nerve relationship.

Materials and Methods

Five Wistar rats were killed by intravenous injection of 1–2 mg kg⁻¹ sodium pentobarbital. The anterior abdominal walls of the rats were carefully isolated by making a deep incision from the costal arches on each side down to the inguinal region and processed using a modified Sihler's technique. The chemical reagents used in this study were obtained from Sigma and Merck Chemical Co. Approval for this experimental study protocol was obtained from the Local Ethical Committee for Animal Studies.

Tissue Preparation

The specimens were fixed by immersion in 10% unbuffered formaldehyde for 1 month. The fixed specimens were washed under running water for 1 h and then macerated in 3% KOH solution (3 drops of 3% H₂O₂ added to 100 ml of the KOH) for at least 3 weeks. The KOH solution was changed daily. The process was considered complete when the specimens appeared transparent or translucent.

The macerated specimens were decalcified using Sihler's solution I (1:1:6 glacial acetic acid:glycerin:1% aqueous chloral hydrate) for 2 weeks, with weekly change of the solution, until the specimens became soft.

Staining

The decalcified specimens were transferred into Sihler's solution II (1:1:6 Ehrlich's hematoxylin:glycerin:1% aqueous chloral hydrate) for 5 weeks. Excess stain was removed from the specimens by placing them in Sihler's solution I. The solution was changed each time it turned purple. This procedure was repeated for 2 days until the muscles were stained light purple and the nerves, a dark color [13].

After excess stain has been removed, the specimens were washed under running water for an hour and then immersed in 0.05% lithium carbonate solution for approximately 1 h. The specimens were cleared in increasing concentrations of glycerin (40, 60, 80 and 100%) containing a few thymol crystals. The specimens were left in each of the solutions for a day until excess stain was removed.

Photography

Glycerin-preserved specimens were trimmed carefully using a binocular microscope to display the nerves, and photographs were taken using Nikon Coolpix 995.

Results

Of the 13 intercostal nerves, the 6–13th individual nerves were followed segmentally to reach the rectus sheath. They were easily traced from lateral (dark-color branches) to medial (light-color branches), as seen in figure 1. As they reached near midline, each individual nerve first gave off lateral and then ventral abdominal branches. The light-colored fine short branches were visible reaching to the abdominal muscles. It was clearly noticeable that the ventral abdominal cutaneous branches traveled to midline by piercing the rectus sheath (fig. 1).

The branches of T₆ and T₇ nerves innervated the skin superior to the umbilicus, T₈ around the umbilicus and T_{9–13} and L₁ inferior to the umbilicus. The light-colored fine short branches within the rectus sheath were motor as well as sensory nerves to the rectus abdominis muscle. The nerve traveling laterally upwards along the linea alba was thought to be cranial iliohypogastric nerve (fig. 1).

Discussion

In recent years, several investigators have successfully applied Sihler's staining technique to demonstrate detailed innervations to various muscles in rabbits [13–16], dogs [17, 18] and humans [19–21].

In this study, in order to highlight the uniqueness of Sihler's staining technique for demonstrating intramuscular nerve fibers, we selected rat anterior abdominal wall to identify and name the nerve branches between the lay-

Fig. 1a, b. Abdominal muscles seen from above. U = Umbilicus; RS = rectus sheath; ICN (T₆–T₁₃ = intercostal nerves (ventral ramie); LACB = lateral abdominal cutaneous branch; VACB = ventral abdominal cutaneous branch; MB = muscular branches; CIN = cranial iliohypogastric nerve.

ers of muscles. The muscles were shown to be innervated by T_{6–13} and L₁, segmentally. The mixed branches of the concerned nerves were identified as ventral, lateral cutaneous ramie, and they were distributed superficially.

As seen in this study, Sihler's staining technique is a suitable technique for demonstrating intramuscular nerve fibers and it enables the identification of nerve branches and terminal endings. This technique may open up new horizons for the study of the muscle-nerve relationship. It would be valuable in understanding normal and abnormal functions of any given organ in clinical cases and/or experimental research models as well as in surgical procedures, i.e. suturing abdominal muscles, or avoiding iliohypogastric nerve trapping during suturing of the abdominal layers [22–25]. In embryological studies, this technique could also help determine the changes in the morphological structure of the nerve distribution during embryonic development.

Conclusion

We presented the nerve distribution of the rat abdominal wall in detail by Sihler's stain technique.

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